

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A01N 63/00	A3	(11) International Publication Number: WO 96/39840 (43) International Publication Date: 19 December 1996 (19.12.96)
(21) International Application Number: PCT/US96/08553 (22) International Filing Date: 4 June 1996 (04.06.96) (30) Priority Data: 08/484,784 7 June 1995 (07.06.95) US (71) Applicant: THE O'DONNELL FAMILY TRUST [US/US]; 1145 Linda Vista Drive, San Marcos, CA 92009 (US). (72) Inventor: O'DONNELL, Boyd; 1145 Linda Vista Drive, San Marcos, CA 92009 (US). (74) Agents: SCHNEIDER, Carol, A. et al.; Lyon & Lyon, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071-2066 (US).		(81) Designated States: CA, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 30 January 1997 (30.01.97)
(54) Title: TREATMENT OF SOIL AND PLANTS WITH A COMPOSITION CONTAINING BACILLUS LATEROSPORUS (57) Abstract A method for treating soil with a composition containing the microorganism <i>Bacillus laterosporus</i> strain BOD is disclosed. Treatment of the soil with <i>B. laterosporus</i> strain BOD results in certain beneficial changes to the soil including maintenance of an alkaline pH, fixation of plant nutrients, neutralization of odors, a reduction in aerobic and coliform bacterial counts, and inhibition of plant pathogenic bacteria and fungi. A method for treating plants with <i>B. laterosporus</i> strain BOD to inhibit the growth of plant pathogenic organisms is also disclosed.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

INTERNATIONAL SEARCH REPORT

International Application No
PL., US 96/08553A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01N63/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,5 055 293 (ARONSON ET AL.) 8 October 1991 see claims	1-11
X	--- JOURNAL OF PHYTOPATHOLOGY, vol. 138, no. 3, 1993, pages 189-208, XP000610785 A.M. ROSALES ET AL.: "Identification of some bacteria from paddy antagonistic to several rice fungal pathogens " see page 189, the abstract see page 190, last paragraph - page 191, paragraph 1 see page 204, paragraph 3 see page 206, last paragraph - page 207 --- -/--	11

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search

28 November 1996

Date of mailing of the international search report

10-12-1996

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Muellners, W

INTERNATIONAL SEARCH REPORT

International Application No

PL./US 96/08553

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PESTICIDE SCIENCE, vol. 37, no. 4, 1993, BARKING, ESSEX, GB, pages 355-363, XP002019401 J.O. BECKER & F.J. SCHWINN: "Control of Soil-borne Pathogens with Living Bacteria and Fungi: Status and Outlook " see the whole document ---	1-11
A	US,A,4 663 162 (KADO ET AL.) 5 May 1987 see column 3, line 54 - column 5, line 5; claims 1-3 ---	1-11
A	US,A,4 952 229 (H.M. MUIR) 28 August 1990 see claims ---	1-10
A	CHEMICAL PATENTS INDEX, BASIC ABSTRACTS JOURNAL Week 8730 23 September 1987 Derwent Publications Ltd., London, GB; AN 87-210719 XP002019403 & JP,A,62 138 380 (BIOSTAR K.K.) see abstract ---	1-10
A	CHEMICAL PATENTS INDEX, BASIC ABSTRACTS JOURNAL Week 8830 21 September 1988 Derwent Publications Ltd., London, GB; AN 88-209768 XP002019404 & JP,A,63 146 723 (SANKYO YUKI KK) see abstract ---	1-10
A	TRENDS IN BIOTECHNOLOGY , vol. 7, no. 2, February 1989, CAMBRIDGE, GB, pages 39-44, XP002019402 J.W. KLOPPER AT AL.: "Free-living bacterial inocula for enhancing crop productivity" see the whole document ---	1-10
A	US,A,4 582 704 (BAKER AT AL.) 15 April 1986 see claim 1 -----	11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/08553

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-5055293	08-10-91	CA-A- 1306670	25-08-92
US-A-4663162	05-05-87	NONE	
US-A-4952229	28-08-90	AU-B- 610200	16-05-91
		AU-A- 1919188	05-04-90
		CA-A- 1235917	03-05-88
US-A-4582704	15-04-86	NONE	

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A01N 63/00	A2	(11) International Publication Number: WO 96/39840 (43) International Publication Date: 19 December 1996 (19.12.96)
(21) International Application Number: PCT/US96/08553 (22) International Filing Date: 4 June 1996 (04.06.96) (30) Priority Data: 08/484,784 7 June 1995 (07.06.95) US (71) Applicant: THE O'DONNELL FAMILY TRUST [US/US]; 1145 Linda Vista Drive, San Marcos, CA 92009 (US). (72) Inventor: O'DONNELL, Boyd; 1145 Linda Vista Drive, San Marcos, CA 92009 (US). (74) Agents: SCHNEIDER, Carol, A. et al.; Lyon & Lyon, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071-2066 (US).		(81) Designated States: CA, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: TREATMENT OF SOIL AND PLANTS WITH A COMPOSITION CONTAINING BACILLUS LATEROSPORUS (57) Abstract A method for treating soil with a composition containing the microorganism <i>Bacillus laterosporus</i> strain BOD is disclosed. Treatment of the soil with <i>B. laterosporus</i> strain BOD results in certain beneficial changes to the soil including maintenance of an alkaline pH, fixation of plant nutrients, neutralization of odors, a reduction in aerobic and coliform bacterial counts, and inhibition of plant pathogenic bacteria and fungi. A method for treating plants with <i>B. laterosporus</i> strain BOD to inhibit the growth of plant pathogenic organisms is also disclosed.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

DESCRIPTIONTreatment of Soil and Plants With a
Composition Containing *Bacillus laterosporus*Related Applications

This application is a continuation-in-part of application Serial No. 08/236,701 filed April 28, 1994, which is a continuation of application Serial No. 07/908,631
5 filed July 1, 1992, now abandoned, which is a continuation of application Serial No. 07/621,603 filed Dec. 4, 1990, now abandoned.

Technical Field

The present invention relates generally to a method
10 for treating soil to improve the soil for agronomic purposes and for other purposes. The present invention also relates to a method of treating plants to control certain plant pathogenic organisms.

Background Art

15 *Bacillus laterosporus* was not previously known for use in treating soil. A previously known method for increasing the alkalinity of the soil is to add lime. The ability to maintain an alkaline soil pH with *Bacillus laterosporus* is the equivalent to the use of tons of lime
20 per acre per year. *Bacillus laterosporus* was not previously known for use in controlling plant pathogenic organisms.

Summary of the Invention

The present invention is directed to methods for
25 treating soil and plants, using a composition comprising the microorganism *Bacillus laterosporus* strain BOD.

In one separate aspect of the present invention, a method of treating soil with *Bacillus laterosporus* strain BOD to maintain an alkaline pH is contemplated.

In a further separate aspect of the present invention, a method of treating soil with *Bacillus laterosporus* strain BOD to reduce the aerobic bacterial count is contemplated.

5 In a further separate aspect of the present invention, a method of treating soil with *Bacillus laterosporus* strain BOD to reduce soil odor is contemplated.

In a further separate aspect of the present invention, a method of treating soil with *Bacillus laterosporus*
10 strain BOD to inhibit plant pathogenic organisms is contemplated.

In a further separate aspect of the present invention, a method of treating plants with *Bacillus laterosporus* strain BOD to inhibit plant pathogenic organisms is
15 contemplated.

Accordingly, an object of the present invention is to provide methods for treating soil and plants with *Bacillus laterosporus* strain BOD.

Detailed Description of the Invention

20 Treatment of soil with a composition containing *B. laterosporus* strain BOD results in a number of beneficial changes for agronomic purposes. The *B. laterosporus* strain BOD that are added to the soil maintain the pH level of the soil in the alkaline range. The ability to
25 perform this function is equal to tons of lime per acre per year. The maintenance of the soil in the alkaline range has a number of advantages. These include fixing soil nutrients by preventing the acidification of the soil and the resulting leaching of nutrients. Ammonia nitrogen
30 which is easily lost into the air is converted in alkaline pH soil to nitrate nitrogen which is fixed in the soil and easily assimilated by plants. Potassium is more insoluble at high pH and therefore less likely to be leached out by rain. The *B. laterosporus* strain BOD was also effective
35 in eliminating odors. This anti-odor effect was probably

due, at least partially, to the maintenance of a more alkaline pH.

The *B. laterosporus* strain BOD composition is also effective in altering the microbial composition of the soil. These alterations include decreasing the overall number of aerobic bacteria. The reduction in bacteria include a reduction in *Salmonella* species, *Klebsiella* species, *Escherichia coli*, *Staphylococcus* species and total coliform species. The reduction of these bacterial populations indicates a reduction in potentially pathogenic microorganisms. The probable mechanism by which these changes occur is through the maintenance of an alkaline pH, which inhibits the growth of acid bacteria. Other possible factors are the production of metabolites by the *B. laterosporus* which interfere with coliform growth or the competition between *B. laterosporus* strain BOD and the pathogenic bacteria.

B. laterosporus strain BOD was originally isolated from a soil sample from Iceland and was selected for further research based on its ability to inhibit pathogenic bacteria in soil and lab test media. The examples described herein utilize *B. laterosporus* strain BOD to treat soil. Table I below shows the results obtained when soil was treated with *B. laterosporus* strain BOD. Treated soil and untreated soil were compared 30 days after treatment.

TABLE I

	ANALYSIS	CONTROL	30 DAYS	% CHANGE
	aerobic bacteria plate count	52x10 ⁶ /gm	30x10 ⁶ /gm	- 42%
5	total coliform bacteria	460/gm	23/gm	- 95%
	E. coli (fecal coliform)	460/gm	<3/gm	- 99%
10	coagulate positive Staph.	23/gm	<3/gm	- 87%
	Salmonella detection	pos./25 gm	neg./25 gm	- 99%
	Klebsiella detection	pos./25 gm	neg./25 gm	- 99%
15	hydrogen ion (pH)	7.75 pH	8.45 pH	+ 9%
	total kjeldahl nitrogen	1,095 ppm	1,235 ppm	+ 13%
	potassium	7,372 ppm	8,319 ppm	+ 13%
20	phosphorous	510 ppm	524 ppm	+ 3%

In another experiment in the laboratory, *B. laterosporus* strain BOD was shown to be effective in inhibiting the growth of certain plant pathogenic bacteria and fungi in culture. The bacteria include *Clavibacter michiganense*,
 25 *Erwinia carotovora*, *Erwinia chrysanthemi*, *Pseudomonas solanacearum*, *Pseudomonas syringae*, and *Xanthomonas campestris*. The fungi include *Aspergillus* species, *Bipolaris* species, *Cephalosporium* species, *Chaetomium* species, *Colletotrichum magna*, *Fusarium oxysporum*, *Penicillium* species,
 30 *Phytophthora cinnamomi*, *Phytophthora citricola*, *Phytophthora citrophthora*, *Phytophthora parasitica*, *Pythium aphanidermatum*, *Pythium ultimum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Verticillium albo-atrum*, *Verticillium dahliae*, and *Verticillium* species.
 35 Because *B. laterosporus* strain BOD was shown to be effective in the laboratory for inhibiting the growth of the above described species, it is expected that the

application of *B. laterosporus* strain BOD to the soil will be effective as a soil treatment to inhibit certain plant pathogenic organisms, including bacteria and fungi, and thereby reduce or eliminate certain plant diseases.

5 Additionally, it is expected that the application of *B. laterosporus* strain BOD directly to plants will be an effective means for controlling certain plant pathogenic bacteria and fungi.

Application

10 *B. laterosporus* strain BOD can be mixed with water and applied to the soil in the normal course of irrigation, including spray irrigation, drip irrigation, or any other means of irrigation. Alternatively, it can be applied independently of irrigation, i.e soil injection, topical

15 application. Effective ranges for application are between about 3.785×10^9 cells per acre and 3.785×10^{12} cells per acre. The most effective range would be expected to be between about 3×10^{10} cells per acre and 2×10^{11} cells per acre. Generally, a relatively high initial application

20 will be followed by periodic applications thereafter, for as long as necessary to maintain the desired effects. The bacteria could also be applied to the soil as a mixture with other soil additives such as fertilizers, herbicides, or pesticides. The bacteria could also be applied in a

25 dry form such as in a powder, either alone or in formulation with other inert or active ingredients.

Best Mode For Carrying Out the Invention

The preferred method and composition for treating soil with *B. laterosporus* strain BOD consists of using *B.*

30 *laterosporus* strain BOD at a concentration of 10 million cells per milliliter of water. The water/bacteria mixture is applied to the soil by irrigation spraying at an initial rate of two gallons per acre and then applied periodically at a rate of one gallon per acre. Therefore,

35 the initial application is with 7.57×10^{10} cells per acre,

and the periodic application thereafter is with 3.785×10^{10} cells per acre. The soil is treated periodically for as long as necessary to maintain the desired effects.

Bacillus laterosporus strain BOD has been deposited
5 for 30 years at the American Type Culture Collection
(ATCC) in Rockville, Maryland and assigned Accession
Number ATCC 55122. The present invention is not to be
limited in scope by the organism deposited, since the
deposited organism is intended to serve only as an example
10 of one strain of *B. laterosporus* that would be effective
in carrying out the invention. The term "*Bacillus latero-*
sporus strain BOD", for the purposes of this invention, is
intended to mean any strain of *Bacillus laterosporus* that
is effective in treating soil and plants as described
15 herein.

Variations on the specific illustrations of the
invention disclosed herein will be apparent to those
skilled in the art, and it is intended that this invention
be limited only by the scope of the appended claims.

Claims

1. A method for improving the quality of soil comprising treatment of the soil with a composition comprising *Bacillus laterosporus* strain BOD.
- 5 2. A method according to claim 1 wherein said treatment further comprises application of *Bacillus laterosporus* strain BOD to the soil in an amount of between about 3×10^{10} cells per acre and 2×10^{11} cells per acre.
- 10 3. A method according to claim 1 wherein said treatment results in an increase in soil pH.
4. A method according to claim 1 wherein said treatment results in a soil pH of between about 7.5 and 8.5.
5. A method according to claim 1 wherein said treatment results in a soil pH of between about 8.0 and 8.25.
- 15 6. A method according to claim 1 wherein said treatment results in a reduction of soil odor.
7. A method according to claim 1 wherein said treatment results in a reduced count of aerobic bacteria.
8. A method according to claim 1 wherein said treatment results in a reduced count of coliform bacteria.
- 20 9. A method according to claim 1 wherein said treatment results in greater control of plant pathogenic bacteria.
10. A method according to claim 1 wherein said treatment results in greater control of plant pathogenic fungi.
- 25 11. A method of treating plants to inhibit the growth of plant pathogenic organisms comprising treatment of the

plants with a composition comprising *Bacillus laterosporus* strain BOD.